REMARKS

Obviousness Issues (pp. 4-15)

The Examiner rejects the claimed subject matter (Claims 11-13) as allegedly obvious over Nissen et al in view of Valverde et al. We begin by quoting part of the prior argument.

"Nissen et al is cited as disclosing a metabolically engineered S. cerevisiae wherein reduced formation of surplus NADH and an increased consumption of ATP in biosynthesis results in decreased glycerol yield. To this end, a mutant S. cerevisiae was produced in which GLN1 encoding glutamate synthetase, and GLT1 encoding glutamate synthetase were over expressed. GDH1 encoding the NADPH-dependent glutamate dehydrogenase was deleted.

"This led to consumption of 1 mol of NADH and ATP per mole of glutamate instead of 1 mol of NADPH, leading to a reduction of surplus formation of NADH, increased ethanol production and decreased glycerol production.

"Nissen et al does not disclose reducing formation of NADH and ATP by the enzymatic activity of a non-phosphorylating dehydrogenase (e.g. GAPN aka GAPDHN). Indeed, as we have previously pointed out Nissen et al does not disclose reducing formation of NADH at all. When Nissen et al speaks of reduced formation of surplus NADH, this is not in fact via the mechanism of reduction of NADH formation, but via provision of a pathway for consuming NADH.

"The Examiner's contention was that because Nissen et al taught that reducing formation of NADH and increasing consumption of ATP resulted in decreased glycerol formation in yeast, it would have been obvious from Valverde et al that this effect could be obtained also by expressing GAPN in yeast to produce 3-phosphoglycerate with production of NADPH rather than NADH + ATP (Office Action of 12th March 2008 at page 12, line 4).

"As we have previously submitted, Nissen et al's teaching is significantly mis-stated by the Examiner. It does not teach reduction in the production of NADH. Rather, it teaches

provision of an NADH consuming reaction path that does not lead to glycerol. This is a different and distinct mechanism for reducing surplus NADH undermines the Examiner's argument, which starts from a false premise."

At pp. 10-11, the Examiner acknowledges this argument but it does not appear to us that she has explained why it may properly be discounted.

New claim 19 requires that the production of NADH by said yeast is reduced as a result of the activity of the second metabolic pathway.

We now continue with the recapitulation of the prior argument, by way of preface to addressing the examiner's counterarguments.

"The situation in respect of NADH formation and surplus NADH formation upstream of glycerol production may be summarized as follows:

(rate of NADH formation) - (rate of NADH consumption) = (surplus NADH formation)

then

Surplus NADH formation → glycerol production

"Nissen et al we agree teaches reduction of the amount of surplus NADH formation. However, it can be seen from the above that this can be achieved in two ways, one can decrease the NADH formation, or one can increase the NADH consumption. The Examiner's statement of the objection indicated that Nissen et al taught to decrease NADH formation, which is we submit untrue. Rather, Nissen et al taught to increase NADH consumption.

"The Examiner's supposed rebuttal of this point, bridging pages 10 and 11 of the current office action, discusses only the reduction in Nissen et al of surplus NADH. We did not dispute

that Nissen et al teaches reduction of surplus NADH. The issue relates to the nature of the mechanism taught by Nissen et al, which we reiterate is not reduction in NADH production.

"The difference between Nissen et al and the claimed invention may be represented as follows:

Nissen et al.	
Before	After
NADH producing reactions	unaffected
NADPH producing reactions	unaffected
NADH consuming reactions	Boosted: GLN1 and GLT1 over expressed, boosting ammonium + 2-oxoglutarate + NADH = glutamate + NAD
NADPH consuming reactions	Reduced: GDH1 (Glutamate dehydrogenase) deleted, cutting ammonium + 2-oxoglutarate + NADPH = glutamate + NADP
Surplus NADH consuming glycerol production	Reduced
Invention	
NADH producing reactions	Reduced: glyceraldehyde-3-phosphate diverted from glyceraldehyde-3-phosphate + NAD = glyceraldehyde-3-phosphate +NADH
NADPH producing reactions	Boosted: GAPN introduced, boosting glyceraldehyde-3-phosphate + NADP = glyceraldehyde-3-phosphate +NADPH
NADH consuming reactions	Unaffected
NADPH consuming reactions	Unaffected

Surplus NADH consuming glycerol production	Reduced

"Secondly, as explained in Nissen et al, one previous strategy for avoiding production of glycerol in yeast was to block the pathway leading to its production by deletion of genes encoding GPD1 and GPD2, but this led to a strain that could not grow under anaerobic conditions (col. 1, page 70). Nissen et al instead choose to drain off surplus formation of NADH by changing the cofactor requirement in amino acid synthesis.

"Clearly, Nissen et al teaches it to be a problem with that earlier strategy that it resulted in a failure to grow under anaerobic conditions. That will have an effect on what a reader of Nissen et al would consider would be an acceptable modification of Nissen et al. The skilled artisan reading Nissen et al would be discouraged by Nissen et al from adopting any steps that would result in failure to grow under anaerobic conditions.

"Valverde et al discloses a metabolically engineered E. coli in which the NAD-dependent glycolytic phosphorylating G3P dehydrogenase GAPDH was deleted and in which GAPN was expressed resulting in the reaction:

Glyceraldehyde 3-phosphate + NADP = 3-phosphoglycerate + NADPH.

"As a result, the E. coli strain was unable to grow anaerobically on sugars but had recovered the ability to grow aerobically on sugars. It also failed to grow on gluconeogenic substrates (acetate + succinate) and showed a lower growth rate than wild type (col. 1, page 155).

"One consequence of the metabolic engineering in Valverde et al is that the resulting E. coli is unable to grow anaerobically, the very problem that Valverde et al were trying to avoid. A skilled artisan would therefore not perceive Valverde et al as offering a teaching likely to be useful in yeast as an alternative strategy for obtaining the objects of

Nissen et al (reduced glycerol and increased ethanol)."

The examiner, on page 11, characterizes the last response as making four arguments (1)-(4). The first is that Valverde's E. coli has decreased growth rate under anaerobic conditions, while Nissen's is fine, so it wouldn't have been obvious to modify Nissen's yeast to include Valverde's GAPN-mediated pathway).

In response, the Examiner states (P12)

> Regarding 1), the fact that Valverde discloses a metabolically engineered *E. Coli* with deleted GAPDH and expressing a functional GAPDHN with reduced growth under anaerobiosis with specific glucose supplemented substrate conditions, does not preclude GAPDHN from being functional in *E. Coli* growing in anaerobic conditions under different metabolic requirements wherein glycolysis may not be the only energy supplying route for the enterobacterium. Indeed, Valverde illustrates in Fig. 3 cited by Applicants reduced but not abrogated growth of *E. Coli* under anaerobic conditions when culture medium is supplemented with succinate plus glycerol rather than glucose, clearly indicating that expression of a functional GAPDHN in *E. Coli* is not the sole variable determining growth of *E. Coli* during fermentation. Further insight into the variable conditions affecting growth of *E. Coli* even in aerobiosis are taught by Valverde at page 156, col. 2, paragraph 3, when the author states "Noteworthy, the GAPDHN-producing clone failed to grow on M63 medium supplemented with gluconeogenic substrates succinate plus acetate, showing in this medium the same pattern as the parental W3CG strain". Therefore, in aerobic or anaerobic fermentations multiple variables account for the growth of the bacterium including the carbon source.

However, Valverde does not teach "different metabolic requirements" which would fully overcome the problem of reduced growth. Hence, the Examiner's burden is to demonstrate that it would have been obvious how to overcome this problem and thus

this "negative teaching" inherent to Valverde.

The second argument was, in the Examiner's words, that "Valverde does not disclose a strain of E. coli engineered to contain GapN without disablement of production of GAPDH". The examiner does not dispute this (OA page 13, line 1). Rather, the examiner contends that GAPDHN can "compete with advantage" over GAPDH. However, it quite clearly is not "competing with advantage" if it is impairing anaerobic growth.

The examiner goes on to state (middle, P13) that anticipation does not require reduction to practice, only an enabling disclosure. That is true, but the examiner appears to have forgotten that the rejection is for obviousness and not for anticipation.

The Examiner alleges that there are Streptococcus strains that have both GAPDHN and GAPDH. This is a reference to p. 157 col. 2, citing reference [21], Boyd et al. This is a 1995 publication, and if that publication in fact provided motivation to express both GAPDHN and GAPDH in yeast, surely such would have been done before applicants' November 2002 priority date[1].

The Examiner emphasizes Valverde et al.'s allusion to a "dual gene" Streptococcal system, but in their own work, they deleted GAPDH and instead expressed GAPN. This was not merely Valverde's preferred embodiment, it was Valverde's only embodiment. If the examiner wishes to rely on Boyd's teachings, she should do so directly.

Point (3), as framed by the examiner, relates to the need

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[1] For the sake of completeness, it appears that Valverde also mentions that photosynthetic cyanobacteria contain both GAPDHN and a kind of GAPDH ($GAPDH_2$) (page 153, col. 21). The "dual" gene system of the cyanobacteria had been disclosed by Valverde back in 1997 (see reference [9]) yet we find no proposals in the art to transfer such a system to yeast.

for a motivation to combine (OA page 13, bottom). The examiner says (top of page 14) that the GAPDHN pathway would reasonably be expected to reduce NADH surplus, etc.

However, assuming arguendo that this is true, it would also have been reasonably expected to impair anaerobic growth absent further modifications, and this in turn would have resulted in a lack of motivation to combine.

Point (4) was that yeast are different from Valverde's E. coli. The Examiner says

> Regarding 4) the Examiner agrees with Applicants that prokaryotic host *E. Coli* and eukaryotic host Yeast are different host systems for expression of heterologous genes. For example, *E. coli* expression host are, in some instances, considered to be unsafe for the production of proteins by recombinant DNA methods due to their production of unacceptable by-products such as toxins. In contrast, yeast is a suitable host organism for the high-level production of secreted as well as soluble proteins, able to perform many of the post translational modifications found on human proteins. However, both *yeast* and *E. Coli* are fermentative microorganism routinely used in the art for expression of foreign recombinant proteins, and industrially valuable products such as polymers, antibiotics, alcohol and others. The choice of which cell is used depends on the project goal and on the prosperities of the protein/product to be produced (Watson et al., Recombinant DNA, pp. 453-455). Clearly, redox enzymes that catalyze synthesis reactions that lead to the formation of industrial important compounds are shared by both *yeast* and *E. Coli* as fermentative microorganism. Thus investigating what glycolytic pathways may be operative in eukaryotic Yeast by investigating one particular enzyme's activity in *E. Coli* as a test system should be reasonably expected to bring insight into the commonly shared glycolytic pathways of *E. Coli* and yeast.

We respectfully direct the examiner's attention to In re Vaeck, 947 F.2d 488, 200 USPQ2d 1438 (Fed. Cir. 1991), wherein the Federal Circuit refused to accept that a person of ordinary skill would consider "cyanobacteria effectively interchangeable with bacteria as hosts for expression of the claimed genes". We think that the claimed yeast are further removed from Valverde E. coli (or Boyd's Streptococcus) than are Vaeck's cyanobacteria.

It is worth noting that we are claiming more than the mere addition of a single heterologous gene to yeast. We are creating a new metabolic pathway that we intend to interact with a native pathway.

If the examiner wishes to rely on the cyanobacteria and streptococcus teachings, we suggest that those references be made of record, warts and all.

We return to recapitulation of the prior argument, as it has continued vitality despite the examiner's counterarguments.

"In the meantime, making the combination suggested by the Examiner does not produce anything falling with the Applicant's claim, because a natural yeast engineered per Valverde et al to remove GAPDH capacity and to insert GAPN activity would not meet the claim requirement for native GAPDH, i.e. it would lack the first metabolic pathway of the claim.

"Furthermore, we pointed out that it should be recognized that Valverde et al has nothing to say regarding the balance of production of any product by the metabolism of the engineered E. coli, let alone of glycerol/ethanol.

"It follows from this that a skilled artisan seeking an alternative technique to that of Nissen et al for improving ethanol production by decreasing glycerol production in yeast would find nothing about that in Valverde et al. He would not therefore be led to consider a combination of these documents. The rejection is essentially based on hindsight and speculation.

"The two teachings lie in very different fields, one being concerned with the metabolism of a yeast and the other with the

metabolism of a bacteria. A skilled person would be unlikely to look for an alternative solution to the problem addressed in Nissen et al relating to yeast, in a teaching confined to the metabolism of E. coli.

"The Examiner contends that Valverde et al presents the skilled artisan with an alternative method of reducing surplus NADH, which the skilled artisan would readily transfer to adapt the teaching of Nissen et al. However, that Valverde et al provides a method for reducing surplus NADH is purely a conclusion drawn by the Examiner and is not something taught by Valverde et al.

"Valverde et al contains no mention of 'surplus NADH' and neither does in discuss any problem or issue connected to the production of surplus NADH or any desire to reduce NADH production. A skilled artisan wishing to find an alternative way of tackling 'surplus NADH' as an issue, whether in yeast or in any other organism, would have no reason to pay attention to Valverde et al. Valverde et al is merely an academic study investigating what glycolytic pathways may be operative in photosynthetic eukaryotes by investigating one particular enzyme's activity in E. coli as a test system. Thus, Valverde et al conclude on page 158 'This metabolic engineering approach has demonstrated the in vivo operation of a non-phosphorylating bypass involving a glycolytic route with no net energy yield that may be functional in photosynthetic eukaryotes and some bacteria'.

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"As previously pointed out, Valverde et al does not teach an alternative route by which one can obtain the same effect taught in Nissen et al. Whilst Nissen et al teaches a way to 'drain off' surplus production of NADH that will otherwise lead to glycerol production, no such draining off mechanism is taught or provided in Valverde et al. Instead, Valverde et al teaches the introduction of an NADPH producing pathway. Accordingly, the proposed combination is not a substitution of like with like.

"Thus, Valverde et al teaches that the ability to metabolize sugar lost via a deletion of GAPDH can be partially rescued (but only under aerobic conditions) by introducing GAPN. According to the Examiner, it is apparent that the catabolic yield of GAPN includes NADPH. This is clearly quite different from draining off surplus NADH by introducing new NADH consuming reactions as in Nissen et al.

"We previously argued also that even if the combination proposed by the Examiner were to be conceived by a skilled reader on the basis of combining Nissen et al and a part of the teaching of Valverde et al as the Examiner proposes, there would have been several reasons not to hold a reasonable expectation that the desired effect would be achieved.

"First, it would have been unknown whether GAPN could be expressed successfully and effectively in yeast.

"Secondly, it would have been unknown whether production of NADPH via expression and activity of GAPN would have any substantial effect on the level of NADH in yeast. Here it should again be borne in mind that the expression of GAPN does not produce operation of the mechanism taught for reducing glycerol yield in Nissen et al. It does not operate to drain off NADH. Rather the hope on which the Examiner's argument depends would be that avoiding one route to the production of NADH by using GAPN to produce NADPH instead would have a material effect.

"This would of course have been completely unknown. First, it would have been unknown to what extent GAPN if expressed successfully in yeast would become engaged in glycolysis when competing with native yeast enzymes. It should be borne in mind that Valverde et al had deleted the E. coli GAPDH so had not even demonstrated that GAPN would have a material effect in E. coli in which the native glycolysis pathway had not been destroyed, let alone that it would be effective in yeast.

"Lastly, as the Examiner has pointed out [previously], a second consequence of metabolizing G3P to 3-PGA via GAPN rather than via GAPDH is that one does not get production of ATP.

However, the effect on the production of glycerol and ethanol in a yeast of this loss of ATP production would have been quite unknown. As seen in the diagram on page 157 of Valverde et al, ATP is required for consumption in the earlier stages of metabolism of glucose. Valverde et al had reported that their engineered E. coli had a decreased growth rate compared to wild type. A skilled reader would have good grounds for expecting that the hypothesized transformed yeast would also have decreased growth rates."

We submit that a skilled reader would not have found it obvious to combine the teachings as proposed and would not have had a reasonable expectation that the combination would achieve the desired end.

Respectfully submitted,

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